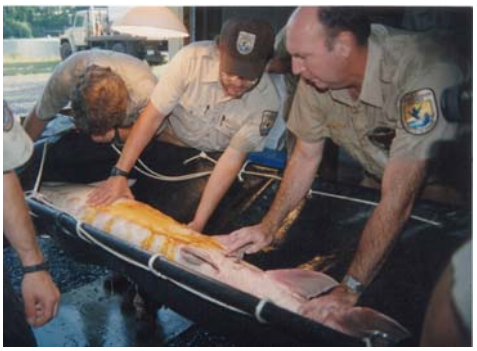
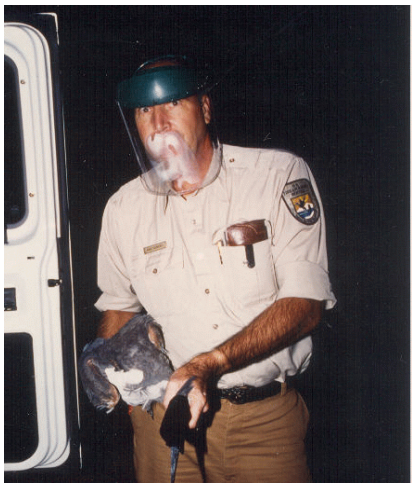
U.S. Fish & Wildlife Service

Northeast Fishery Center
Lamar, Pennsylvania

Annual Report of Biological Activities 2002





The Northeast Fishery Center (NEFC) dedicates this 2002 Report of Biological Activities to Center Director, Mike Hendrix, who retired this year after having dedicated 37 years to the U.S. Fish & Wildlife Service. Mike began his NEFC Directorship in 1989 and much was accomplished toward stewardship of our aquatic resources under his guidance. In Mike's last year on the job, we completed the conversion of an existing building into a genetics laboratory which will provide Region 5 with advanced DNA technology capabilities in support of aquatic species restoration programs. Advanced population ecology studies were also performed which consisted of a hooking mortality project associated with recreational angling of American shad in the Hudson River under a cooperative agreement with the State of New York. In addition, we performed an assessment of the survival of hatchery-reared endangered stocks of Atlantic salmon in Maine using a new fish-marking technology. Applied research continued in many areas of fish culture technology with inter-jurisdictional species such as American shad, Atlantic salmon, and Atlantic sturgeon. In support of restoration efforts for endangered freshwater mussels, NEFC spawned and reared a surrogate mussel species for use in an experimental marking study. Finally, the experimental ozone water treatment plant which was completed the previous year, was successfully tested for it's efficiency in removing pathogens from our water supply. We proudly report the following Biological Activities for 2002:

STUDIES PERFORMED

Study Number and Title:

(Previously unreported results from 2001 experiments):

LM-01-02 Analysis of lipofuscin pigment concentrations in brain tissue from progressive size classes of horseshoe crabs (*Limulus polyphemus*) as a possible indicator of age.

(Current fiscal year, 2002 studies):

LM-02-01 Whole-tray calcein marking of non-feeding Atlantic salmon fry.

LM-02-02 American shad tank spawning - Effects of stocking density, male : female ratio, and efficacy of hormone implant use in males.

LM-02-03 (Pilot study) Induction and non-lethal detection of calcein marks in the Eastern elliptio freshwater mussel Elliptio complanata

LM-02-04 Analysis of various tissues of yearling Atlantic salmon for residual calcein fluorescence after osmotic induction of a calcein mark.

LM-02-05 Comparison of mortality between calcein-marked and unmarked Atlantic salmon fry stocked in the Sheepscot River, Maine (2nd year study).

LM-02-06 Mortality associated with catch and release angling of striped bass in the Hudson River.

LM-02-07 Cryopreservation of Atlantic salmon semen using five extender and cryoprotectant combinations

LM-02-08 Reductions in bacterial micro-organisms by filtration and ozonation of the surface water supply at the USFWS Northeast Fishery Center

OTHER BIOLOGICAL AND RELATED INVESTIGATIONS PERFORMED:

LM02A Fish Health Inspection/Monitoring/Diagnostic Services

LM02B Licensing agreement for calcein detection devices

LM02C Ozone water treatment plant testing

LM02D Participation in the National Wild Fish Health Survey

LM02E Incidence and Prevalence of Infectious Salmon Anemia virus (ISAv) in Sea Run Penobscot River Atlantic Salmon held at Craig Brook NFH for Broodstock

LM02F Participation in Maine Fish Health Advisory Board concerning Infectious Salmon Anemia (ISAv) Issues

LM01G U.S. Fish and Wildlife Service Fish Health Procedures Handbook

LM02H Quality Assurance/Quality Control for Infectious Salmon Anemia virus (ISAv) Samples and Diagnostic Techniques

LM01I Fish Health Extension Services

LM02J Investigative New Animal Drug (INAD) permit exemption for calcein

LM02K U.S. Fish and Wildlife Service Fish Health Policy

LM02L Incidence and Prevalence of Spring Viremia of Carp (SVC) Virus in a watershed of Virginia/North Carolina

STUDIES IN WHICH THE CENTER COOPERATED:

The ecology of whirling disease *(Myxobolus cerebralis*) in Pennsylvania. *Adam Kaeser, Pennsylvania State University*

Effect of temperature, oxygen, dietary phosphorus, and vitamin D3 on phosphorus levels in effluent from experimental culture of rainbow trout. - *Reli Coloso, Department of Pharmacology and Physiology, UMDNJ - New Jersey Medical School, Newark, NJ*

Description of the surface water filtration and ozone treatment system at the Northeast Fishery Center. - *S. Summerfelt and J. Bebak-Williams, The Conservation Fund, Freshwater Institute - Shepherdstown, WV and D. Creaser, U.S. Fish & Wildlife Service, Region 5 Engineering - Hadley, MA*

Horeshoe crab tagging and tag return survey in the Delaware Bay.- *Sheila Eiler, US Fish & Wildlife Service - Maryland Fisheries Resource Office, Annapolis, MD.*

PUBLICATIONS:

Mohler, J.W., M.J. Millard, and J.W. Fletcher. 2002. Predation by captive wild brook trout on calcein-marked versus nonmarked Atlantic salmon fry. North American Journal of Fisheries Management 22:223-228

TECHNICAL INFORMATION LEAFLETS:

LM-02-01 Analysis of Atlantic salmon tissues for residual calcein fluorescence after osmotic induction of a calcein mark using SE-MARK™.

LM-02-02 Producing fluorescent marks on Atlantic salmon fin rays and scales with calcein via osmotic induction.

LM-02-03 Reductions in bacterial micro-organisms by filtration and ozonation of the surface water supply at the USFWS Northeast Fishery Center.

TECHNICAL REPORTS:

Fletcher, J.W. and M. J. Millard. 2002. Effect of hormone selection and water salinity upon tank spawning performance of American shad. In Susquehanna River Anadromous Fish Restoration Committee. Restoration of American Shad to the Susquehanna River, Annual Progress Report. U.S. Fish and Wildlife Service, 2001. Harrisburg, PA.

Millard, M.J., J. Mohler, A. Kahnle, A. Cosman, K. Hattala, and W. Keller. 2001. Mortality associated with catch and release angling of striped bass in the Hudson River. Report to: NY State Dept. of Environmental Conservation, Hudson River Fisheries Unit, New Paltz, NY.

FORMAL PRESENTATIONS:

Barbash, Patricia - Reductions in bacterial micro-organisms by filtration and ozonation of a creek water supply at the USFWS Northeast Fishery Center. Bioengineering Symposium at the American Fisheries Society Annual Conference. August 19, 2002. Baltimore, MD.

Barbash, Patricia - AFS/FHS and USFWS Joint efforts in establishing QA/QC program for fish disease diagnostic laboratories 4th International Symposium on Aquatic Animal Health. Sept. 1-5, 2002. New Orleans, LA

Fletcher, John - Effect of hormone selection and water salinity upon tank spawning performance of American shad. Oxytetracycline Task Force Meeting, January 28 -29, 2002. Richmond, VA

King, Kim - Use of epi-fluorescent microscopy and special stains to determine the presence of lipofuscin in the horseshoe crab Limulus Polyphemus brain, as a possible indicator of age. Poster presentation at the American Fisheries Society meeting. Aug 12-16, 2002. Baltimore, MD.

Millard, Michael. Mortality associated with catch and release angling of striped bass in the Hudson River. American Fisheries Society meeting. Aug 12-16, 2002. Baltimore, MD.

Mohler, Jerre - Field evaluation of calcein marks on Atlantic salmon fry stocked into the West Branch Sheepscot River, Maine. Maine Technical Advisory Committee Atlantic Salmon Research Forum. Jan. 16. University of Maine.

Mohler, Jerre - Overview of the N.E. Fishery Center. Lycoming County Chapter meeting of the Audubon Society. Jan. 23. Williamsport, PA.

Mohler, Jerre - Field evaluation of calcein marks on Atlantic salmon fry stocked into the West Branch Sheepscot River, Maine. Poster presentation at the 2002 Region 5 Project Leader Conference. Feb. 25 - Mar.1. Norfolk, VA.

Mohler, Jerre - Calcein marking, a promising new tool for hatchery product evaluation. 53rd Northwest Fish Culture Conference. Dec. 3-5. Bellingham, WA.

NEFC Staff - Northeast Fishery Center staff provided walking tours of the facility to Region 5 Project Leaders and other guests. Focus areas included: Lamar Fish Health Center, Genetics Laboratory, Intensive Culture Building, Ozone Water Treatment Building, Dissolved Gases Laboratory, and Experimental Sturgeon Rearing Pond. June 13.

National Committee participation:

Carta, Anthony.-Served as NEFC representative in Service and Maintenance Management System (SAMMS). NEFC was one of 3 fishery units nationally selected to pilot test the MAXIMO® software.

Coll, John and Patricia Barbash.- Served on the National Fish Health Policy Revision Committee to re-write the National Fish Health Policy and Procedures handbook.

Coll, John.- Served on the National Title 50 Revision Committee to re-write Title 50 regulations for importing fish into the U.S.

Jodun, Wade.- Served as NEFC representative in Service and Maintenance Management System (SAMMS). NEFC was one of 3 fishery units nationally selected to pilot test the MAXIMO® software.

Millard, Michael.- Served on the U.S. Atlantic Salmon Assessment Committee, the technical body which responds to Atlantic salmon assessment tasks defined by the U.S. section to North Atlantic Salmon Conservation Organization (NASCO).

Millard, Michael. - Atlantic States Marine Fisheries Commission: member of horseshoe crab Technical Committee, and serves as chair of horseshoe crab Stock Assessment committee.

Millard, Michael. - Served as team leader for the Chesapeake Bay Susquehanna River Ecosystem team.

Selmer-Larsen, Kim.- Served on the Great Lakes Disease Committee to represent Region 5 relative to disease issues affecting the Great Lakes.

Other Significant Committee Participation:

Barbash, Patricia.- Served on the Maine Fish Health Advisory Board to make recommendations to the Maine commissioners relative to fish health issues impacting wild Atlantic salmon populations and commercial aquaculture.

Barbash, Patricia.- Served on the New England Salmonid Health Committee to make recommendations to the New England Atlantic Salmon Commission (NEASC) relative to fish health issues impacting the New England states.

Study Number: LM- 01-02

Title: Analysis of Lipofuscin pigment concentrations in brain tissue from progressive size classes of horseshoe crabs Limulus polyphemus, as a possible indicator of age.

Principal Investigator: Kim King, Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: Stew Michels, Delaware Natural Resources and Environmental Conservation (DNREC),
J. R. and C. McConaughay Old Dominion Univ.- VA; Vicki Blazer, US Geological Survey/Biological Resources Division (USGS/BRD), Leetown-WV

Background and Justification:

Recently, much attention has centered on the management of the horseshoe crab fishery, due to overexploitation, the need for a sustainable biomedical fisheries and the importance of horseshoe crab eggs as a food source for migratory shore birds. Available data necessary to quantitatively assess the status and dynamics of the population is uninformative and not well developed. Standardized data collection such as spawner and egg count surveys, fishery catch and effort, estimates of natural mortality rates, growth rates, and fecundity, is critical to guide the management decisions and policies which will serve to protect the species. In January of 2000, the Horseshoe Crab Stock Assessment Committee (SAC) described and recommended appropriate population dynamic models and assessment surveys to meet these data needs. Due to the current inability to age Limulus, the catch-survey model, applied to species whose age structure is unknown, was recommended. The SAC notes that the development of a reliable aging technique may permit alternative, perhaps better assessment techniques for the future. Presently, various subjective field observations are used to approximate the age of juvenile and adult horseshoe crabs. Data collected from observations include, the number of mating scars on the shell and the extent of shell erosion, presence/absence of trabeculae, size of dorsal spines and posterior projections of the prosoma (fused head and thorax) and opisthosoma (segmented posterior portion), and the number of annual growth rings in the shell of epibionts attached to adult Limulus. The ability to age crabs accurately is essential to completely understand the dynamics of the horseshoe crab population. Old Dominion University, is currently researching a possible aging technique for the Delaware Bay blue crab using a histological procedure to determine the number and size of lipofuscin granules in the olfactory lobe of the crab brain and has been successful in correlating lipofuscin concentrations with the size of the animal. It has been shown that size and number of lipofuscin granules in the olfactory lobe of the American lobster and carapace lengths were significantly related to age. Large lipofuscin concentrations have been found in several areas of the horseshoe crab brain, particularly in the anterior ganglions. These results suggest the possibility that the lipofuscin technique, if standardized, can differentiate cohorts in natural populations of the horseshoe crab.

Study Objectives:

Quantify the variability present in lipofuscin concentrations in brain tissue from progressive size classes of horseshoe crabs.

Materials and Methods:

Horseshoe crab specimens were collected from Delaware by Stew Michels, Delaware Natural Resources and Environmental Conservation (DNREC), and preserved in 10% formalin. Brain tissue samples were obtained from 200 crabs, approximately 20 females per size class. Size was classified as carapace width. Two laboratories, Old Dominion University, Norfolk Virginia and the Northeast Fishery Center, Lamar, PA divided up the raw materials and simultaneously conducted standardized processing to quantify lipofuscin concentration. The dissection process and histological procedure was conducted according to accepted, published procedures. Image analysis and quantification of lipofuscin was conducted at the USGS/BRD Leetown laboratory.

Results

1) Numerous lipofuscin granules were found throughout the corpus pedunculata of Limulus polyphemus. The granules in this area were large and well defined with fluorescence and histochemical staining techniques.

2) Sudan Black B proved to be the most effective staining method for lipofuscin quantification.

3) Further research should evaluate the age-dependence of lipofuscin in horseshoe crabs and the relationship between lipofuscin and body size within known age classes to determine if the lipofuscin technique can be used to differentiate age cohorts in natural populations.

Study Number: LM-02-01

Title: Whole-tray calcein marking of non-feeding Atlantic salmon fry.

Principal Investigator: Jerre W. Mohler; Northeast Fishery Center
Co-Invest/Cooperators: NEFC biologists

Background and Justification:

In spring 2001, NEFC tested mass-marking of non-feeding ATS fry with calcein by immersing entire heath trays in a concentrated calcein solution. The same immersion parameters were used as previously employed for marking small batches of fry. Results showed that fry became labeled with calcein but the marks were not as brilliant as expected compared to fry which were marked in small batches in previous years' trials. Limited numbers of fry precluded further testing of various immersion parameters to improve calcein mark quality when applied to whole trays of fish. The 2001 whole-tray immersion technique was also applied to 30,000 ATS fry at Craig Brook NFH that same spring where fry were marked and stocked out into the West Branch Sheepscot River, ME. Field recovery of marked vs. unmarked fry in the fall of 2001 resulted in about 43% marked vs. 57% unmarked fish recovered. Researchers noticed that some marks were difficult to determine in the field. Therefore more testing is needed to improve initial mark application concerning whole-tray immersion techniques. We propose to manipulate immersion parameters on domestic ATS fry hatched in 2002 to improve mark quality on fish subject to whole-tray marking techniques. Resulting information will be transferred to field stations using this marking strategy so that marked fish which are recaptured in the field will have greater and more consistent mark readability.

Study Objectives:

In March, 2002, at least five Heath trays containing approximately 5,000 fry each (domestic White River NFH stock) will be exposed to five immersion procedures to attempt improvement of calcein mark quality over that which was achieved in 2001.

Materials and Methods:

In November, 2001, the NEFC crew spawned resident domestic White River ATS broodstock and collected about 35,000 eggs. Pooled, unfertilized (green eggs) were enumerated using three 50-egg displacement samples then grouped into 6,000 - 7,000 egg lots. Each lot was fertilized in native ovarian fluid with 5-10mls of raw milt. After fertilization, eggs were water-hardened for 30 minutes in 100ppm active iodine solution and placed into Heath trays at a volume of one lot per tray. Water flow through incubators was maintained at about 19L/min.

When larvae reached a Developmental Index of about 85, marking procedures were initiated using the following treatments:

	Salt bath	Rinse	Calcein bath
Control -	5% salt (3.5 min)	momentary	0.5% (3.5 min)
A -	5% salt (3.5 min)	none	0.5% (3.5 min)
B -	5% salt (3.5 min)	none	0.5% (7.0 min)
C -	5% salt (3.5 min)	none	0.5% (3.5 min)**
D -	5% salt (3.5 min)	momentary	0.5% (3.5 min)**

** fry were constantly stirred with a turkey feather during salt and calcein immersions

Intensity of calcein marks were determined with a battery-powered calcein detection device and were scored subjectively for level of fluorescence.

Results

At 48-h post treatment, mortality in all experimental lots was less than 10 fish. The most brilliant fluorescence was seen from fish in treatments B and C which were equivalent in mark quality. This suggests that a freshwater rinse between salt and calcein immersions may be counter-productive to producing the optimum calcein mark fluorescence when using this technique to label Atlantic salmon fry. In addition, stirring the fry gently with a feather while in the calcein solution for 3.5 min yielded fluorescent marks equivalent to those seen in fry which were not stirred but remained in the calcein solution for twice the amount time (7 min).

Study Number: LM-02-02

Title : American shad tank spawning - Effects of stocking density, male : female ratio, and efficiency of hormone implant use in males

Principal Investigator: John Fletcher, Jerre Mohler, and Mike Millard, NEFC

Co-Investigators/Cooperators: Mike Hendrix, Kim King, Wade Jodun, and Pat Farrell, NEFC ; Dick St.Pierre, Susquehanna River Coordinator, Mike Hendricks, Pennsylvania Fish and Boat Commission; Ray Bleistine, Normandeau Associates; and Richard Bradshaw, Syndel International, LTD.

Background and Justification

In 1998, the Northeast Fishery Center began a cooperative effort to develop and conduct tank spawning technology to establish self-sustaining populations of American shad (AMS) imprinted to the West Branch of the Susquehanna River and to augment egg production for Pennsylvania Fish and Boat Commission (PFBC). Annual goals were to refine tank spawning methodology, to provide 5 to 10 million fertilized shad eggs for PFBC, Van Dyke Shad Hatchery, and to stock one to two million oxytetracycline (OTC) marked fry in the West Branch drainage of the Susquehanna River. Tank spawning technology development at the NEFC, as reported by Fletcher and Millard (2002), has determined that AMS captured and transported to NEFC for spawning are subject to a series of stressors which impact survival and reproduction. It has been determined that hormone implanted American shad in the NEFC tank spawning system, survive and produce a program sufficient number of eggs (>24,000) per female when water quality is maintained at 3 ppt salinity, 18.3 C, and 8.0 mg/L dissolved oxygen.

Study Objectives

In the Spring of 2002 we will: (1) determine the effect of stocking ratios of male to female AMS as measured by: adult survival, number of eggs produced per day per available female, and egg viability, (2) determine the effect of brood stock density in spawning tanks as measured by: adult survival, number of eggs produced per day per available female, and egg viability, (3) determine the effect of Salmonid Gonadotropin Releasing Hormone (SGnRHa) implant use in male shad (with vs without) as measured by: adult survival, number of eggs produced per day per available female, and egg viability, and (4) provide 5 to 10 million AMS eggs to PFBC

Method and Materials

NEFC received 1200 shad over a six week period collected at the West Lift of Conowingo Dam (total 1200). Two brood stock sex ratios were evaluated:2.5 **:** 1 (male:female) and 1.5:1. Two stocking densities were established :1 fish/104 L or 1fish/224 L respectively. Upon arrival, all female AMS received a cholesterol based implant containing 150 ug SGnRHa - Ovaplant ® S 150 Syndel Laboratories Ltd., into the dorsal musculature. Males for one of the two tanks only received implants. Egg collection for each shipment focused on capture of primary production pulse which occured typically 2 to 3 days post implant. Reproduction was evaluated in terms of viable eggs produced per female on any given day. When daily production exceeded 5 L, eggs were transported and incubated at Van Dyke Shad Hatchery. Spawns of less than this amount were incubated at NEFC and stocked into Bald Eagle Creek. A tank spawning system utilizing flow-through ambient Susquehanna River water was employed at Conowingo Dam.

Results

Total and viable egg production per female were significantly increased ($P \leq 0.05$) in tanks where male AMS received SGnRHa implants. Results indicate that continued use of hormone implants for male AMS would be beneficial to tank spawning operations. AMS stocked at rates of 1/104 L and 1/217 L did not produce statistically different total numbers of eggs per female. Greater production of viable eggs per female was observed for the lower density ($P = 0.10$). No effect upon reproductive success was found between stocking ratios of 2.5 or 1.5 males per female, in terms of eggs per female or viable eggs per female. Production costs for hormone implants may be reduced by 29% by utilizing the stocking ratio of three males to two females *vs*. five males to two females. NEFC stocked 51,350 fry into Bald Eagle Creek, West Branch of the Susquehanna River and provided 8.0 million eggs to the Van Dyke Shad Hatchery.

Study Number: LM-02-03

Title: (Pilot study) Induction and non-lethal detection of calcein marks in the freshwater mussel Elliptio complanata

Principal Investigator: Jerre W. Mohler-Northeast Fishery Center
Cooperators: Bill Lellis - USGS, BRD-N. Appalacian Res, Lab- Wellsboro, PA

Background and Justification:

As a result of the magnitude and immediacy of the nationwide threats to native freshwater mussel fauna, the National Native Mussel Conservation Committee (NNMCC) was formed comprising individuals from state and federal resources agencies, the commercial mussel industry, academia, and the Nature Conservancy. To begin addressing needs for mussel conservation, the group produced a conservation document titled: National Strategy for the Conservation of Native Freshwater Mussels which contains various strategies for reaching conservation goals. Included are recommendations to determine the viability of artificially-propagated juveniles to determine their suitability for release in restoration and recovery plans. But in order to evaluate suitability, comparisons on growth and survival must be made with individuals from wild populations. This necessitates some type of marking technique to permit differentiation between hatchery-reared and wild mussels. Batch-marking with chemicals would be less labor intensive than conventional tagging techniques and could reduce the need for culturing juveniles for an extended period of time. One group of chemical marks which has received increased attention are those produced from fluorochromes. These compounds have the ability to label bony or calcified tissues of organisms and can be detected as visible fluorescence under certain optical conditions. The most commonly used fluorochromes are oxytetracycline and calcein. Of these two, calcein has shown promise to provide an additional marking tool having capabilities of providing fisheries evaluations not possible or practical to perform with previous marking techniques. NEFC has been performing experimental fish marking with calcein since 1995 and has developed a calcein mark detector (SE-MARK^{TM} - Patent FWS-3667) which allows the operator to discern between marked and non-marked fish by the presence or absence of a visible green fluorescence in calcified structures. This pilot study will evaluate the ability of the calcein detection device to differentiate between marked and unmarked hatchery-reared juvenile Elliptio complanata mussels. In addition, application of the calcein mark via an osmotic-induction technique was evaluated.

Objectives

Beginning in the fall of 2002, we determined the efficacy of osmotic induction and 24-h static immersion to induce calcein marks using approximately 500 Elliptio complanata. freshwater mussels.

Methods

The study took place at NEFC in the summer of 2002. Juvenile Elliptio mussels were obtained by collecting and spawning wild adults using juvenile lake trout from Allegheny National Fish Hatchery for host fish. After transforming on the gills of host fish, mussel glochidia were placed into screened containers supplied with sand for substrate and given flow-thru water from NEFC's reservoir. After reaching an age of 60 days, marking procedures began with a test of 4-minute salinity tolerance at either 0.5, 1.0, or 2% salinity. The highest, safe level was then used in the osmotic induction treatment for calcein marking. Calcein marking proceeded with four treatments: (1) osmotic induction using 0.5% calcein solution (2) 24-h static immersion using 250mg/L calcein (3) control for osmotic induction and (4) control for 24-h static immersion. Each treatment was limited to 1 batch due to limited numbers of juveniles. Individuals were processed in small screened containers and immersed into marking solutions contained in shallow stainless steel pans. After marking was completed, batches were placed back into a fresh water supply for at least 24 hours prior to mark evaluation.

Results

Approximately 1950 transformed glochidia were collected over a 15-d period beginning 12 d after host lake trout were infected. About 500 juveniles were recovered after a 60-day period and were used in the marking study. The maximum no-observed-effect salinity tolerance for 4 minutes was determined to be the 1%salt level. At 90-d post-marking, overall survival was best in the osmotic treatment group at 48% vs. 28% in 24-h static treatment. No visually-discernable difference in brilliance of calcein mark fluorescence was found between treatments . Control treatments had overall 90-d survival of 40% for the osmotic treatment which received a salt bath only while 24-h controls had 50% survival. Total length at 90 d was similar between all treatment groups and ranged from 0.58 - 1.00mm. Calcein fluorescence was not readily visible using the SE-MARK^{TM} detector due to small size of the organisms, therefore assessment was done with fluorescence microspcopy.

Study Number: LM-02-04

Title: Analysis of various tissues of yearling Atlantic salmon for residual calcein fluorescence after osmotic induction of a calcein mark.

Principal Investigator: Jerre W. Mohler-Northeast Fishery Center
Cooperators: Ron Secor - Western Chemical, Ferndale, WA

Background and Justification

Fisheries restoration and recovery plans often rely on some type of fish marking to evaluate progress toward achieving management goals. There are currently numerous types of tagging and marking options available to fishery managers, but each has it's own limitations; therefore the search for additional tagging and marking technology continues. The fluorochrome compound commonly known as calcein has shown promise to provide an additional marking tool having capabilities of providing fisheries evaluations not possible or practical to perform with previous marking techniques. It has been evaluated in laboratory experiments as a method of marking fish otoliths as well as fin rays, scales, and other calcified tissues. In order to fully evaluate the usefulness of this emerging technology, an Investigative New Animal Drug (INAD) permit must be obtained from the US Food and Drug Administration (FDA) so that fishery managers and other investigators can legally mark and release fish for testing purposes. Obviously, it is important to understand the effects which any chemical or drug has on the target organism as well as any effect on humans concerning a potential food item. Part of this understanding involves gaining knowledge concerning any residence time the drug has in various fish tissues once applied. With this study we aim to evaluate the presence of residual calcein in blood, dorsal musclulature, liver, kidney, and spleen from yearling Atlantic salmon which have been immersed in calcein using the osmotic induction techniques developed at NEFC.

Objectives

In 2002, we marked 40 Atlantic salmon yearlings with calcein using osmotic induction techniques and evaluated various tissues for calcein residues via fluorescence microscopy at various intervals up to 72 hours post-application.

Methods

The study took place at NEFC in 2002. Approximately 40 yearling Atlantic salmon of Connecticut River domestic origin were used in the study. Individuals were batch-marked by immersion in 1.5% NaCl solution for 3.5 minutes followed immediately by immersion in a 0.5% calcein solution using the protocol for this procedure developed at NEFC. After marking procedures were complete, fish were placed into culture tank supplied with flow-through freshwater and cared for in a manner similar to other captive fish. Calcein used in this study was supplied by Western Chemical and was representative of that manufactured under Western Chemical's INAD permit for drug sponsorship.

After 1 hour in fresh water, 5 fish were randomly removed from the tank for tissue sampling. The fish were sacrificed and target tissues were sampled (blood, dorsal muscle, kidney, liver, spleen). Sampled tissues were examined via fluorescence microscopy for the presence of calcein and digital photos were taken as necessary. Fluorescence was scored as present or absent in tissues. Subsequent fish were sampled at 24, 48, and 72 hours post calcein-application and tissues were evaluated as previously described. If fluorescence was found in any tissues after 72 hours, addition samples were taken at 24-hour intervals.

Results

The lowest level of visually-detectable fluorescence for SE-MARK^{TM} calcein was found to be $1X10^{-3}$ ppm in the sample viewed without a cover slip and $1X10^{-2}$ ppm in the sample with the cover slip. Blood and muscle tissue were devoid of visually-detectable fluorescence caused by calcein residues at the 10d post-treatment sample period and beyond. This suggests that in the yearling salmon tested, muscle and blood tissue had calcein residue levels between 10^{-2} and 10^{-3} ppm during the same sample period post-exposure when the visual detection limits are applied. Using known tissue percentages, we find that one individual contains less than 5.6×10^{-9}g of calcein residue in muscle tissues at this stage of development. By extrapolation, we then calculate the level of residue at less than 1.2×10^{-10} g of calcein per gram of muscle tissue (less than 1.2 parts per ten-billion) when the fish attains a total length of about 20 cm (8 in). Applying the lowest level of visually-detectable calcein found in serial dilutions with the cover slip would result in the estimation of less than 1.2×10^{-9} g of calcein per gram of muscle tissue in the 8-in fish (less than 1.2 parts per billion).

Study Number: LM-02-05

Title: Comparison of mortality between calcein-marked and unmarked Atlantic salmon fry stocked in the Sheepscot River, Maine (2nd year study)

Principal Investigators: Mike Millard, Jerre Mohler - Northeast Fishery Center
Co-investigators:; David L. Perkins-R5; Tom King-Craig Brook Natl Fish Hatchery

Background and Justification

Millions of Atlantic salmon fry are stocked throughout river basins in Maine each year. This requires a tremendous effort on the part of state and federal agencies, as well as the many volunteers that assist each year. A major obstacle to evaluating the performance of these fry has been the inadequacy of existing technologies for marking fry; however, recent advances in the use of calcein to produce an externally-visible mark now offers a solution.

An important question that must be answered before use of new marking techniques is whether marking causes any direct and indirect mortality. In 2001, a total of about 60,000 calcein-marked and non-marked Atlantic salmon fry reared at CBNFH were field-evaluated for survival in the fall of 2001 after being stocked as non-feeding fry in the Sheepscot and Narraguagas Rivers, Maine. Field recovery and data assessment showed that marked and non-marked fry were recovered at the expected 1:1 ratio. This study represents a second year of field assessments. Results from the study will help assess the utility and practicality of using calcein to mark fry as part of the monitoring and evaluation program of Atlantic salmon recovery in Maine.

Study Objectives

A total of about 50,000 calcein-marked and non-marked Atlantic salmon fry reared at CBNFH were field-evaluated for survival in the fall of 2002 after being stocked as non-feeding fry in the Sheepscot and Narraguagas Rivers, Maine. Age 1+ parr from the previous years' study will also be captured and assessed for calcein marks.

Materials and Methods

The study was conducted in the West Branch of the Sheepscot River The majority of fry (about 50,000) were stocked into the W. Branch Sheepscot. Fry were stocked according to normal practices except that half of all fry stocked at each location were calcein-marked according to whole-tray immersion procedures developed and tested by NEFC in 2002. Fry marked as part of this study were stocked according to standard practices. In early fall, age-0 fish and 1+ parr were electrofished from multiple sites in the West Branch Sheepscot River using backpack shocking units and the number of marked vs. unmarked fish were compared. After each pass through a sampling area, captured fish were anesthetized with AQUI-S, an iso-eugenol anesthetic regulated by the US Food and Drug Administration under an Investigative New Animal Drug Permit exemption (INAD). Anesthetized fish were examined with a battery-operated calcein detection device to discern marked vs. non-marked fish. All fish were released back into the sampling area as soon as data have been collected. Study-wide, numbers of marked and non-marked fry captured from each sampling location were compared using a Replicated Goodness of Fit test (G-statistic) (Sokol and Rohlf 1981) to test the hypothesis that marked and non-marked fry have survived from stocking date to capture date at a 1:1 ratio.

Results

Seven sites yielded sufficient numbers (n= >5) of YOY to perform the replicated G-test. Out of these 7 sites, three showed calcein-marked vs. unmarked fish were recovered at the expected 1:1 ratio ($P < 0.05$). The other four sites yielded ratios in favor of unmarked fish with 2 sites being highly skewed. These 2 highly-skewed sites resulted in an overall recovery ratio of 3:1 in favor of unmarked YOY which obviously did not fit the expected 1:1 ratio. In light of the 1:1 ratio found in the previous years' study and coupled with the fact that 3 out of 5 sites this year did conform to the expected 1:1 recovery, we are not certain why the overall 2002 ratio was skewed in favor of non-marked YOY. Tissue samples from non-marked YOY are undergoing DNA analysis to determine whether any non-marked fish resulted from natural reproduction. Out of 52 age 1 or greater fish captured, thirteen fish were found which had retained the calcein mark from the previous year's release (2001 stocking).

Study Number: LM-02-06

Title: Catch and Release Mortality Rates in the Striped Bass and American Shad Fisheries of the Hudson River

Principal Investigators: Mike Millard - Northeast Fishery Center
Co-investigators: Andrew Kahnle and Kathryn Hattala -NY State Department of Environmental Conservation **(NYSDEC)**

Background

Catch and release sport fishing commonly occurs in many fisheries, such as the striped bass (STB) and American shad (AMS) fisheries of the Atlantic Coast. The contribution of catch-and-release practices to overall fishing mortality is often not estimated. Estimates from the recreational fishery survey indicated that over 16 million and 15 million STB were released in 1997 and 1998, respectively. Assuming an 8% hooking mortality rate for STB translates to over 1.2 million mortalities each year. These estimates exceed the 1997 and 1998 commercial harvest. We reviewed results from previous hooking mortality studies, however results from studies conducted in one river system, or in different environmental conditions, may not be directly transferable to other geographic areas; therefore rates of hooking mortality of Hudson River STB and AMS may differ from those reported in other areas. In addition, many factors can increase hooking mortality, such as: hook type, bait type, salinity, water temperature fish size, and hook location. A preliminary study conducted jointly by the USFWS and NYSDEC in the spring of 1999 indicated that mortality for STB and AMS was about 30% for both species. This project addressed several difficulties identified in the preliminary study and refined the estimates for differing gear types. The results will provide NYSDEC fishery managers with information necessary to determine the contribution of hook and release mortality to the Hudson River STB and AMS fisheries. Results will be useful in developing guidelines for reducing mortalities from hook-and-release angling on the Hudson River.

Study Objectives

(1) to estimate the mortality of STB and AMS associated with catch and release practices commonly occurring in the spring recreational fisheries in the Hudson River, and (2) to assess the influence of selected variables, e.g. water temperature, playing and handling time, hook location, degree of bleeding, and length, on hooking mortality rates.

Methods

The STB study occurred in 3 sampling weeks during May 2001 near Kingston, NY. The AMS study occurred during the peak of the spawning run in 2002, at the tailwaters of the dam at Troy, NY. Fish were caught with spinning gear consistent with that used in the Hudson River STB and AMS fisheries. In addition to USFWS and NYSDEC staff, volunteer recreational fisherman were recruited. After a fish was angled, a uniquely numbered tag was applied to the dorsal fin. Fish were then transported in aerated live wells to onshore tanks. For stripers, the effect of hook type (conventional “J”-hook vs. circle hook) on mortality was investigated. Playing and handling time, hook location, degree of bleeding, and hook type were recorded for each fish caught. Gender and age were determined after the observation period. Control fish were electrofished from the river, but were handled, tagged, transported, and retained similar to angled fish. Each holding tank contained similar numbers of angled and control fish and were held for 5 days. Dead fish were removed and recorded daily. Selected dead angled fish were necropsied to determine cause of death. Data analysis occurred during November and December of each sample year.

Results

Striped bass - Participating anglers contributed 159 STB during the 13 angling days and 143 control fish were captured via electrofishing. Mortality of control fish was low at 2.8% within the 5-day observation window, whereas 16.3% of the angled fish died. Most anglers were unwilling to employ circle hooks, consequently only 37 STB were captured on them. The mortality rate for circle hooks was 5%, whereas that for J-hooks was 16% but hook type was not statistically related to mortality of angled STB. Hook location and the occurrence of bleeding were influential in determining probability of death of an angled fish. All fish (n=7) that swallowed the hook, exhibited bleeding and died. **American shad** A total of 485 AMS were caught via angling, and 8 (1.6%) of these died within the 5-day observation period. Nine (3.9%) of the 233 control fish died. The 1.6% mortality of angled fish fell within the 95% confidence interval for mortality expected due to handling alone (1.2% - 6.6%). These results suggest that mortality associated with hook and release of American shad, as practiced in our study, may be essentially nonexistent.

Study Number: LM-02-07

Title: Preliminary investigations of cryopreservation of Atlantic salmon milt using six extender-cryoprotectant combinations

Principal Investigator: Kim King, Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: John W. Fletcher and Jerre Mohler, NEFC; Bill Wayman, Warm Springs Fish Technology Center

Background and Justification

The Atlantic salmon (ATS) Restoration Program relies heavily upon fish cultural facilities to produce fry, parr, and smolts for restoration stocking. Despite stocking efforts, sea-run returns of Atlantic salmon continue to be at very low levels (U.S. Atlantic salmon Assessment Committee 2002). Concurrently, in the Connecticut River program, the returning female to male ratio is skewed approximately 2 to1. To address this issue, the U. S. Fish and Wildlife Service has taken strong interest in the potential of cryopreservation techniques to maximize the conservation of available ATS male genetic material.

Cryopreservation of milt from commercially farmed salmonids has been reported by the reviews of Scott and Baynes (1980 and Stoss and Donaldson (1982). Although much of the work has been done with rainbow trout, preservation of Atlantic salmon milt has been achieved using vials (Truscott and Idler 1969; Mounib 1978) or pellets (Stoss and Refstie 1983). However, there are presently no routine techniques available for sperm storage in Atlantic salmon using the plastic French straws widely used for bovine reproduction.

Study Objectives

This report describes some preliminary trials examining the cryopreservation of Atlantic salmon milt using six extender-cryoprotectant combinations employing the French straw method.

Materials and Methods

Milt was collected from eight individually identified Atlantic salmon at the NEFC and shipped on ice overnight in oxygenated ziplock bags to Warm Spring Technology Center for cryopreservation. Milt from individual males was combined with one of the following six extender-cryoprotectant combinations: Cloud-5% Dimethyl sulfoxide (DMSO), Cloud-10% DMSO, Hanks Balanced Salt Solution (HBSS)- 5% DMSO, HBSS- 10% DMSO, Gallant-5% DMSO, Gallant-10% DMSO. Diluted milt was immediately drawn into 0.5 ml French straws and placed directly into dewars containing liquid nitrogen and shipped back to the NEFC. On the day of spawning, control milt was collected from the same males and held in oxygenated ziplock bags on ice prior to fertilization. Eggs were collected and pooled from twenty Atlantic salmon females and stored in oxygenated bags in a covered bowl in a water bath (8EC) prior to fertilization. Frozen milt was thawed at 40EC in a water bath for seven seconds and immediately used upon thawing. One straw was used to fertilize approximately 200 eggs. Eggs were then placed in randomly assigned compartments in Heath trays and incubated. Mortalities were enumerated and removed periodically during incubation. After eggs developed to the eyed stage, they were physically shocked, and survival was determined by percent eye-up. Fishers Least Significant Difference test was used to determine the significance of study results.

Results

Fertilization rates, expressed as mean percent eyed eggs for cryopreserved Atlantic salmon milt, ranged from 0.3 (Gallant-10%DMSO) to 4.9 (Cloud 5%DMSO). The control treatment showed a significantly greater eye-up at 82.2 % than all other treatments. However, the Cloud-5% DMSO treatment at 4.9% eye-up resulted in a greater fertilization rate than all other cryo-protectants tested ($P<0.05$).

Study Number: LM-02-08

Title: Reductions in bacterial micro-organisms by filtration and ozonation of the surface water supply at the USFWS Northeast Fishery Center

Principal Investigator: Patricia Barbash - Northeast Fishery Center (NEFC)
Co-Invest/Cooperators: John Fletcher, Anthony Carta - NEFC; Steve Summerfelt, Julie Bebak-Williams, Conservation Fund Freshwater Institute; Duncan Creaser, U.S. Fish and Wildlife Service, Region 5 Contracting and General Services

Background and Justification

Surface water use in flow-through aquaculture systems poses a risk of contamination with microorganisms which are known fish pathogens. Exposure to these pathogens by fish maintained under intensive culture conditions to these pathogens often leads to infection, clinical disease, and losses in production. The only sure way to rid a facility of a pathogen, once introduced, is to depopulate infected fish and disinfect the entire facility. An example of a fish culture facility contaminated with pathogens from its surface water source is the Intensive Culture (IC) Building at the Northeast Fishery Center in Lamar, PA (NEFC). Losses in production and research specimens due to disease and parasite outbreaks indicated the necessity to disinfect the surface water source. A water filtration and ozonation system was installed to treat the surface water used in the IC building.

Study Objectives

Our objective was to assess the efficacy of the treatment system to inactivate bacterial organisms present in the surface water from Fishing Creek. Variables tested included: water flow, turbidity, temperature, ozone concentration, contact times, and presence or absence of pathogenic bacteria.

Materials and Methods

Water Quality Sample Methods.- Water was collected from "pre-filtered" and "post-ozonation" locations of the ozone treatment system. These water samples were tested immediately for dissolved ozone concentration using a Palintest® dissolved ozone test. Water samples were also tested for turbidity. *Bacterial Enumeration*.- Number of heterotrophic bacteria in water samples was determined including the fish pathogens: Aeromonas salmonicida, Yersinia ruckeri, Flavobacterium columnare and F. psychrophilum. At each sample site, five replicates were established in sterile collection containers. Pre-filtration water samples were diluted depending upon turbidity of the water at the time of the sample. Post-ozonation water samples were not diluted. A total of 100 mL of each sample replicate was then filtered through a sterile membrane filter (0.45 micron pore) mounted on a disinfected filter apparatus. After filtration, the filter was removed from the apparatus using sterile forceps, and placed on selective media for 5-10 minutes. *Bacterial Isolation and Identification.-* Coomassie Brilliant Blue agar (CBB) was used for isolation and quantification of heterotrophic bacteria as well as selective identification of Aeromonas salmonicida. Tryptone yeast gelatin (TYG) agar was utilized for selective identification of yellow pigmenting bacterial species. Bacterial identifications were performed on two of the sample dates to determine the dominant species of bacteria entering the ozone treatment system and those surviving the treatment. Identifications were accomplished on CBB plates from representative colonies isolated onto TSA. Isolates were identified and those which stained Gram positive were incubated for several additional days and observed microscopically for development of spores as a general way to identifiy Bacillus species. Yellow pigmenting bacteria were isolated on TYG and tested for growth on TSA to identify non- F. columnaris and F. psychrophilum yellow pigmenters.

Results:

No pathogenic bacterial organisms were observed in any water samples during this study. Heterotrophic bacteria were inactivated by ozonation by over 99.3%. Yellow pigmenting bacteria enumerated in the pre-filtered water samples were inactivated at or above 98.5% post-ozonation. Disinfection by ozonation accomplished 2 to 4 $\log_{(10)}$ reductions in surface water bacteria per mL of water regardless of flow, turbidity, temp. and ozone contact time. Actual numbers of bacteria from post-ozonated samples were often a fraction of a cfu/mL. Inactivation of bacteria exceeded 99% even when microscreen filtration was taken off-line. Complete (100%) disinfection of both heterotrophic and yellow pigmenting bacteria was observed when ozone contact time was recorded at the highest test value of 21.3.

OTHER BIOLOGICAL AND RELATED INVESTIGATIONS PERFORMED:

LM01A Fish Health Inspection/Monitoring/Diagnostic Services. - The Lamar Fish Health Center processed 340 laboratory cases in fiscal year 2002. Region 5 has a very extensive fish health monitoring program to enhance the fish health inspections, allowing continual surveillance of the health status of the stocks, some of which have been identified as very limited distinct population segments (DPS) which the Service has listed under the Endangered Species Act (ESA). The Fish Health Center had 36 inspection cases, which included 16 that were conducted, as outlined in the Service Fish Health Policy, as virology lab services only for non-Service entities. These statistically based fish health examinations are essential to prevent the spread of fish diseases through fish and/or egg transfers and are necessary to enable facilities to comply with regulations on transporting and releasing fish. In addition to the 158 monitoring cases involving examination of fish, 4 Service facilities provided 50 water monitoring cases, where water from rearing units was examined by the water filtration method, a very effective proactive protocol for diagnosing furunculosis before an epizootic occurs and for evaluating efficacy of ozone disinfection. In fiscal year 2002 seventeen laboratory cases were diagnostic examinations, where moribund fish were examined and tested to determine the cause(s) of mortalities and other problems and recommendations for resolution were provided. As a cooperator in various fisheries projects within the region, the Fish Health Center also examined aquatic invertebrates (6 cases) which involved fisheries management activities with sandworms, bloodworms, and freshwater mussels and their potential to spread fish pathogens.

LM01B Licensing agreement for calcein detection devices.- In 2000, NEFC submitted a patent application for both a bench-top and hand-held calcein detection device which will make it feasible to quickly and efficiently detect fluorescent marks on fish under rigorous field conditions without the need for a microscope. In 2001, negotiations with Western Chemical Co. were undertaken by the USFWS for granting exclusive licensing to Western Chemical to produce and market the devices. An agreement was signed in Feb., 2002 which represents the first time the USFWS has successfully negotiated this type of agreement for a patented device (FWS Patent #3667).

LM01C Ozone water treatment plant testing.- Construction was completed on the ozone water treatment plant for the Intensive Culture Unit in 2001. Region 5 Fisheries, Engineering, Contracting and the Freshwater Institute of Shepardstown, WV, reviewed and discussed design, costs, benefits and potential application of ozone treatment systems. Engineering and fish health aspects were also reviewed.

LM01D Participation in the National Wild Fish Health Survey. -This project, launched in 1997, continues to involve all nine Service fish health centers nationwide incorporating standardized diagnostic techniques and data management methods to ensure comparability. In fiscal year 2002, the Fish Health Unit initiated 70 cases for the Survey, in which 2,668 fish (25 different species) from a total of 58 sites were examined and efforts continued to enter completed cases into the NWFHS database. This database which is capable of single and double queries based on either fish species or fish pathogens, is now accessible via the Internet on the service website. The National Wild Fish Health Survey is partnership driven and fiscal year 2002 enabled the Lamar Fish Health Center to conduct cooperative work with Stratus Consulting and the New York Ecological Services Field Office on contaminants in the Hudson River; Penn State University on whirling disease in feral trout populations in Pennsylvania; Shenendoah National Park on wild, unmanipulated population inventories; Maryland DNR on assessing the prevalence of largemouth bass virus in bass resident in several lakes; and several state natural resource agencies on developing broodstocks from feral populations. Outreach activities to increase awareness of the Survey and involve other Fish and Wildlife Service programs included demonstrations of the database on the Internet to the Region 5 Regional Directorate Team as well as development of a NWFHS Outreach Committee coordinated out of the Washington Office.

LM01E Incidence and Prevalence of Infectious Salmon Anemia virus (ISAv) in Sea Run Penobscot River AtlanticSalmon held at Craig Brook NFH for Broodstock. -Due to the intensive efforts of state and federal agencies to control Infectious Salmon Anemia virus (ISAv) in Atlantic salmon in maritime culture in waters of Maine, a surveillance protocol for screening sea-run Penobscot River Atlantic salmon as they are captured and brought to Craig Brook National Fish Hatchery was initiated to determine incidence in this population. A sub-sample (124) of fish were sampled non-lethally (blood)and tested by reverse transcriptase-polymerase chain Reaction (RT- PCR) and cell culture on SHK-1 and ASK cells. All fish tested negative by both PCR and cell culture techniques. As a tool for managing this virus at the facility, the entire population (n=383) was similarly screened, following cohabitation and prior to spawning. All tested negative and no isolation / quarantine of eggs was deemed necessary.

LM01F Ongoing Participation in Maine Fish Health Advisory Board concerning Infectious Salmon Anemia virus(ISAv) Issues.- The Maine Fish Health Technical Committee serves as a scientific advisory board to the state Commissioners. The group, containing a representative from the Lamar Fish Health Center, is very involved with Infectious Salmon Anemia virus (ISAv), as well as other fish health issues related to private aquaculture and wild resources. The Center has established a monitoring program for all sea- run Atlantic salmon mortalities as has the Maine salmon industry which began participation in a US Dept. of Agriculture (USDA), Animal Plant Health Inspection Service (APHIS) Indemnification Program for eradication of ISAv in Maine. Part of the two year program involves depopulation and fallowing of all salmon net pens in Cobscook Bay, where ISA has been a problem for the private aquaculture industry for over a year. Due to the low numbers of the distinct population segments (DPS) of Atlantic salmon listed under the Endangered Species Act, it is vital that the impacts of this and other diseases are held to a minimum. The unresolved issue which remains is the inconsistent management of ISAv across international borders in Canada, leaving the Maine sites along the border vulnerable to disease. The MFHTC continues to seek solutions to management of ISAv in Atlantic salmon wild and cultured stocks through communication with private industry and international regulators.

LM01G U.S. Fish and Wildlife Service Fish Health Procedures Handbook. -In cooperation with all eight other USFWS fish health centers, and in collaboration with the American Fisheries Society - Fish Health Section, a procedural manual for Fish Health Inspection Protocols has been completed. A representative of the Lamar FHC chaired subcommittees to gather and edit procedures for the Sampling, Bacteriology and Quality Assurance Chapters of this Manual. The intent of the Handbook is to establish a nationally consistent set of protocols for use by all fish health inspectors and diagnostic laboratories when performing fish health inspections of fish culture and aquaculture facilities. The document will be provided to the Fish Health Task Force of the Congressional Joint Subcommittee on Aquaculture (JSA) for their review and adoption as a national standard for Fish Health Inspections. The document has also become a chapter in the updated version of the AFS/FHS “Bluebook”. It is available for viewing on the Internet at http://fisheries.fws.gov/FWSFH/NFHSmain.htm

LM01H Quality Assurance/Quality Control for Infectious Salmon Anemia virus (ISAv) Samples and Diagnostic Techniques. -The Lamar Fish Health Center participated in an ISAV assay and procedure QA/QC program with National Marine Fisheries Service University of Maine at Orono, and MicroTechnologies, Inc. during fiscal year 2002. Receiving blind samples collected by UMO from clinical and subclinical experimentally infected fish, as well as negative fish, this exercise is examining the differences between sampling blood (non-lethal) and tissues (kidney/spleen) as well as the accuracies and sensitivities between the PCR and cell culture assays, all conducted with duplicate samples in two laboratories. A total of 60 fish, producing 360 assays were completed at the Lamar Fish Health center and MicroTechnologies Inc. The study identified problems with detection of the virus by cell culture. A repeat of the study is planned for FY2003, to include the Atlantic salmon kidney (ASK) cell line.

LM01I Fish Health Extension Services -The Lamar Fish Health Center continues to provide extension services to all federal, state, tribal and private inquiries in the area of fish health. Services provided include verbal consultations, provision of supplies for fish necropsies, treatment recommendations and calculations, antibiotic injections, vaccinations, and provision of procedural protocols.

LM02J Investigative New Animal Drug (INAD) Permit Exemption for Calcein - NEFC cooperated with the Bozeman Fish Technology Center (USFWS National INAD Office) in Bozeman, MT; the US Fish & Wildlife Service -Ecological Services Office in State College, PA; and Western Chemical, Inc. of Ferndale Washington to provide experimentation, literature searches, and other assistance as part of an application to the U.S. Food and Drug Administration for an Investigative New Animal Drug exemption permit to begin using calcein as an experimental new drug for marking fish.

LM02K U.S. Fish and Wildlife Service Fish Health Policy - The Lamar Fish Health Center participated in a Service-wide effort to revise the Service's national policy on fish health, 713 FW 1-5. One of the major changes is the removal of technical laboratory procedures from the policy, with reference to a Service Procedures Manual. This will allow procedures, which are subject to change due to advances in research, to be updated in the manual, without a formal (administrative) policy revision. Chapter 1 provides the general scope and responsibilities, with the most eminent change being the reinstatement of Whirling Disease as a pathogen of concern. Chapter 2 addresses operations, where special cases, i.e endangered species, are considered in regard to different sample sizes and methodologies (non- lethal). Likewise, Chapter 3 covers exotic disease eradication, where destruction of any fish may be exempted from the provisions upon consent of the assembled task force that pathogen containment is achieved. Chapter 4 establishes a national consistency of how services to non-Service entities is handled, providing discretion based on available resources. Finally, an entirely new addition to the Policy, Chapter 5, addresses feral populations and the Service Controlled Propagation Program. A quantitative risk assessment grid has been developed to be used to evaluate disease risks in the development of the fish health management plan, as required by the Controlled Propagation Policy. For the first time, this document has received both internal and external review and comment, enabling Service partners to assist in addressing fish health concerns.

LM02L Incidence and Prevalence of Spring Viremia of Carp (SVC) Virus in a watershed of Virginia/North Carolina - The Lamar Fish Health Center, in cooperation with the Warm Springs Fish Health Center and North Carolina WRC and Virginia DGIF, performed investigation of free- ranging cyprinids in the Dan River watershed from which the first isolation of SVCV in North America occurred at an aquaculture facility. Various techniques, including cell culture for isolation of the virus and antibody testing of serum for determining exposure were conducted, with several instances of fish with antibody titers occurring. Due to the seasonality of the expression/detection of this virus, further cooperative investigations are planned for Spring of 2003.

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